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Abstract: OBJECTIVE This in vitro study aimed to investigate the preventive effect of brushing with anti-erosive toothpastes compared to a conventional fluoride toothpaste on dentine erosion. MATERIALS AND METHODS Bovine dentine specimens (n=12 per subgroup) were eroded in an artificial mouth (6 days, 6×30 s/day) using either citric acid (pH:2.5) or a hydrochloric acid/pepsin solution (pH:1.6), simulating extrinsic or intrinsic erosive conditions, respectively. In between, the specimens were rinsed with artificial saliva. Twice daily, the specimens were brushed for 15 s in an automatic brushing machine at 2.5 N with a conventional fluoride toothpaste slurry (elmex, AmF) or toothpaste slurries with anti-erosive formulations: Apacare (NaF/1% nHAP), Biorepair (ZnCO3-HAP), Chitodent (Chitosan), elmex Erosionsschutz (NaF/AmF/SnCl2/Chitosan), mirasensitive hap (NaF/30% HAP), Sensodyne Proschmelz (NaF/KNO3). Unbrushed specimens served as control. Dentine loss was measured profilometrically and statistically analysed using two-way and one-way ANOVA followed by Scheffe's post hoc tests. RDA-values of all toothpastes were determined, and linear mixed models were applied to analyse the influence of toothpaste abrasivity on dentine wear ($p<0.05$). RESULTS Dentine erosion of unbrushed specimens amounted to 5.1±1.0 m (extrinsic conditions) and 12.9±1.4 m (intrinsic conditions). All toothpastes significantly reduced dentine erosion by 24-67% (extrinsic conditions) and 21-40% (intrinsic conditions). Biorepair was least effective, while all other toothpastes were not significantly different from each other. Linear mixed models did not show a significant effect of the RDA-value of the respective toothpaste on dentine loss. CONCLUSION Toothpastes with anti-erosive formulations reduced dentine erosion, especially under simulated extrinsic erosive conditions, but were not superior to a conventional fluoride toothpaste.

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Prevention of dentin erosion by brushing with anti-erosive toothpastes

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Keywords: dentin, erosion, toothpaste

ABSTRACT

Objective: This in vitro study aimed to investigate the preventive effect of brushing with anti-erosive toothpastes compared to a conventional fluoride toothpaste on dentin erosion.

Materials&Methods: Bovine dentin specimens (n=12 per subgroup) were eroded in an artificial mouth (6 days, 6x30 s/day) using either citric acid (pH:2.5) or a hydrochloric acid/pepsin solution (pH:1.6), simulating extrinsic or intrinsic erosive conditions, respectively. In between, the specimens were rinsed with artificial saliva. Twice daily, the specimens were brushed for 15 s in an automatic brushing machine at 2.5N with a conventional fluoride toothpaste slurry (elmex, AmF) or toothpaste slurries with anti-erosive formulations: Apacare (NaF/1% nHAP), Biorepair ($ZnCO_3$-HAP), Chitodent (Chitosan), elmex Erosionsschutz (NaF/AmF/$SnCl_2$/Chitosan), mirasensitive hap (NaF/30% HAP), Sensodyne Proschmelz (NaF/KNO_3). Unbrushed specimens served as control. Dentin loss was measured profilometrically and statistically analysed using two-way and one-way ANOVA followed by Scheffe`s post-hoc tests. RDA-values of all toothpastes were determined, and linear mixed models were applied to analyse the influence of toothpaste abrasivity on dentin wear ($p<0.05$).

Results: Dentin erosion of unbrushed specimens amounted to 5.1±1.0µm (extrinsic conditions) and 12.9±1.4µm (intrinsic conditions). All toothpastes significantly reduced dentin erosion by 24-67% (extrinsic conditions) and 21-40% (intrinsic conditions). Biorepair was least effective, while all other toothpastes were not significantly different from each other. Linear mixed models did not show a significant effect of the RDA-value of the respective toothpaste on dentin loss.

Conclusion: Toothpastes with anti-erosive formulations reduced dentin erosion, especially under simulated extrinsic erosive conditions, but were not superior to a conventional fluoride toothpaste.

INTRODUCTION

The use of conventional fluoridated toothpastes was shown to be of limited efficacy in the prevention of erosive tooth wear compared to the application of high-concentrated fluoride products, especially when applied at high frequency.[1,2] However, compared to sodium fluoride-containing toothpastes experimental toothpastes containing other fluoride compounds, e.g. titanium tetrafluoride or stannous fluoride, were shown to be of higher efficacy.[3,4]

In the last years, numerous toothpastes with “anti-erosive” or “repairing” claims were marketed, which contain other active ingredients besides fluorides, e.g. hydroxyapatite nanoparticles, zinc-carbonate-hydroxyapatite nanoparticles, potassium nitrate, stannous salts, chitosan and/or proteins.

Previous studies showed that some of these toothpastes presented a higher protective potential on enamel erosion than conventional toothpastes, [5,6,7,8,] while other studies failed to show this effect. [9,10,] Interestingly, it was also demonstrated that the erosion-inhibiting potential of both conventional and anti-erosive toothpastes was higher when enamel specimens were just exposed to rather than brushed with the respective toothpaste slurries.[9,11] In the latter case, it is possible that brushing hampers or removes surface precipitates essential for the anti-erosive effect. It is therefore still questionable whether patients at risk for dental erosion really benefit from the use of these anti-erosive or repairing toothpastes compared to conventional fluoride toothpastes.

So far, most studies tested the erosion-protective effect of toothpastes with special formulations mainly on enamel specimens. However, considering that dentin is frequently exposed by erosion, especially in high risk patients suffering from eating disorders,[12] reflux[13] or alcohol abuse,[14] prevention of dentin erosion by home-use oral care products is becoming increasingly important.

Therefore, the aim of the study was to investigate the preventive effect of brushing with anti-erosive toothpastes compared to a conventional fluoride toothpaste on dentin erosion by simulated extrinsic and intrinsic erosive conditions. The null-hypothesis was that the erosion protective effect of anti-erosive or repairing toothpastes on dentin is not superior to a conventional fluoride toothpaste.

MATERIALS & METHODS

Specimen preparation

Specimen preparation for erosive cycling

A total of 216 cylindrical dentin specimens (diameter: 3 mm) were prepared from the root surfaces of freshly extracted, non-damaged bovine incisors, which were stored in 0.1% thymol solution until they were used. The dentin specimens were prepared using a water-cooled trephine bur and embedded in acrylic resin blocks (Paladur, Heraeus Kulzer, Germany) each containing three dentin specimens. The unique shape of the resin blocks with a round tip on one end and a cornered tip on the other allowed exact repositioning of the specimens in the brushing machine as well as in the profilometer. The specimens were ground flat with water-cooled discs (1200, 2400 and 4000 grit, Water Proof Silicon carbide Paper, Struers, Erkrath, Germany) and randomly allocated to 16 groups with n = 12 specimens each.

Specimen preparation for RDA measurement

Sixty-four roots of freshly extracted bovine incisors were used. In preparation for the RDA-measurement, the root surfaces were polished under water-cooling (Sof-Lex discs, 15 µm and 3 µm, International Dental Supply, Hialeah, FL, USA) for two minutes with 40-60 g load.[15] The specimens were then radiated (Atomic Institute of Vienna, Austria) and randomly allocated to 7 test groups (toothpastes) and one group with the standard ISO abrasion reference material (each group n = 8 roots).

Solutions and toothpastes

Erosive cycling was performed using either citric acid (pH: 2.5) or hydrochloric acid/pepsin solution (pH: 1.6), simulating extrinsic or intrinsic erosive conditions, respectively. Citric acid at pH 2.5 was obtained by adding 2.81 g/l citric acid (Fluka, Buchs, Switzerland) to 1 liter of distilled water. The hydrochloric acid/pepsin solution was prepared by dissolving 5 mg/ml NaCl in distilled water and adjusting the pH to 1.6 with hydrochloric acid (chemicals from Merck, Darmstadt, Germany). Subsequently, 1.5 mg/ml pepsin (4800 U/ml; P-6887, pepsin from porcine gastric mucosa, 3200 U/mg, Sigma–Aldrich, Seelze, Germany) was added to the HCl solution.[16]

The artificial saliva used was prepared following the formulation (0.002 g ascorbic acid, 0.030 g glucose, 0.580 g NaCl, 0.170 g $CaCl_2$, 0.160 g NH_4Cl, 1.270 g KCl, 0.160 g NaSCN, 0.330 g KH_2PO_4, 0.200 g urea, 0.340 g Na_2HPO_4 in 1000 mL distilled water) given by Klimek et al.[17] and was renewed each day.

The toothpaste slurries were prepared by mixing artificial saliva and the respective toothpastes (Apacare (NaF/1% nHAP), Biorepair ($ZnCO_3$-HAP), Chitodent (Chitosan), elmex (AmF), elmex Erosionsschutz (NaF/AmF/$SnCl_2$/Chitosan), mirasensitive hap (NaF/30% HAP), Sensodyne Proschmelz (NaF/KNO_3)) in the ratio of 3:1. The pH-values of the toothpaste slurries were measured with a pH meter (Metrohm, Herisau, Switzerland).

The composition of the toothpastes and pH-values are described in Table 1.

Experimental Procedure

Erosive cycling was performed for 6 days in artificial mouth, previously presented by Wiegand et al. Fehler! Verweisquelle konnte nicht gefunden werden. Six times daily, the specimens were eroded with the respective acid solution for 30 s at 2 ml/min flow rate. Between the erosive cycles (60 min), the specimens were rinsed with artificial saliva at 0.5 ml/min flow rate. Overnight, the specimens were stored in artificial saliva.

Each day 1 h before the first and 1 h after the last erosive attack, the specimens were brushed for 15 s with a toothpaste slurry in an automatic brushing machine applying reciprocating linear motion to the toothbrushes (ParoM43, Esro AG, Thalwil, Switzerland). The brushing machine was adjusted to a constant brushing frequency of 60 strokes/min and a constant brushing load of 2.5 N. During brushing, the right and left sides of the specimens were covered with a stainless steel foil (0.1-mm thick) leaving a 2-mm wide area in the middle of each specimen exposed for brushing and reference areas prevented from brushing abrasion for the profilometric analysis.

RDA measurement

Relative dentin abrasion of the toothpastes was determined by brushing the specimens in a sandwich technique previously described by Imfeld.[15] Irradiated root specimens were first brushed with the reference standard, then with the experimental product and then again with the reference standard. In each step, a manual toothbrush (Paro M43, Esro AG, Thalwil, Switzerland) was moved over the roots perpendicular to the longitudinal axis for 25 min at a rate of 60 cycles/minute at 2.5 N.

For the standard abrasive slurry, silica (Sident®, Evonik Degussa GmbH, Essen, Germany) was mixed with carboxymethylcellulose, glycerol and a saliva substitute stock solution. The slurries of the experimental toothpastes were made from a mixture of saliva substitute, bi-distilled water, and sodium bicarbonate with the respective toothpaste.[15]

After each brushing sequence, ^{32}P radiation activity was measured in the slurries relative to the reference slurry (Phosphor-Imager®, Molecular Dynamics, Sunnyvale, CA, USA).

Profilometry

Dentin loss was analyzed profilometrically using a mechanical contact profilometer (Perthometer S2, Mahr, Göttingen, Germany). From each specimen, 5 baseline surface profiles were recorded (Perthometer S2, Mahr, Göttingen, Germany) with a distance of 100 µm between each profile. The profiles were obtained by moving the diamond stylus across the dentin surface and the references areas perpendicularly to the direction of the movement of the toothbrush. This assessment was done at baseline and after completion of the experiment. All resin blocks had a notch, which fits the metal jig of the profilometer table preventing the rotation of the specimen and allowing repositioning and exact matching of the baseline profiles with the respective final erosion profiles. The reproducibility of the repeated measurements was checked previously, and the vertical difference of each profile with regard to the first profile was found with a range of 0 ± 0.031 µm.[18]

Calculation was done using the software of the profilometer (Mahr Perthometer Concept 7.0, Mahr, Göttingen, Germany) as previously described in detail.[18]

Statistical analysis

Dentin loss (mean ± standard deviation) of all groups was calculated. Normal distribution of the data was tested using Kolmogorov–Smirnov and Shapiro-Wilk tests. As data were normally distributed, dentin loss was analysed using two-way and one-way ANOVA followed by Scheffe`s post-hoc tests ($p < 0.05$). Mean (± standard deviation) RDA-values of all toothpastes were computed.

Linear mixed models with dentin loss as dependent variable, RDA-value and erosion condition as covariates and toothpaste as random effect was applied to analyse if dentin loss is depending on the RDA-value of the respective toothpaste.

RESULTS

Dentin loss of the unbrushed specimens amounted to 5.1 ± 1.0 μm (extrinsic conditions) and 12.9 ± 1.4 μm (intrinsic conditions). The conventional toothpaste reduced dentin loss significantly ($p \leq 0.000$) by 45% (extrinsic conditions) or 37% (intrinsic conditions). The anti-erosive toothpastes reduced dentin loss significantly ($p \leq 0.037$) by 23 to 67% and 22 to 40%, respectively, but were not superior to the conventional AmF toothpaste (Table 2).

RDA-values of all toothpastes are also presented in Table 2. Linear mixed models did not show a significant effect ($p = 0.079$) of the RDA-value of the respective toothpaste on dentin loss.

DISCUSSION

Most of the anti-erosive or repairing toothpastes reduced dentin loss significantly, but were not significantly superior to the conventional AmF toothpaste, thus, the null hypothesis is accepted.

In the present study the erosion-protective effect of the toothpastes was analysed under both simulated extrinsic and intrinsic acid exposure. Dietary acid exposure at a high frequency was simulated by erosion with citric acid at a pH commonly found in erosive beverages. Endogenous acid exposure was simulated by erosive demineralisation with hydrochloric acid at a pH usually present in the stomach and by the addition of pepsin, which led to an additional enzymatic degradation of collagen.[20] Due to the lower pH and the enzymatic degradation of the organic matrix, simulated intrinsic erosive conditions were more aggressive and thus, provoked higher dentin loss than simulated extrinsic conditions.

Specimens were brushed twice daily under standardized conditions following recent recommendations for erosion/abrasion studies.[21] To simulate a realistic scenario, the specimens were brushed twice daily for a short period (15 s). Even under these severe erosive conditions, the use of a conventional fluoride toothpaste seems sufficient for patients with dentin erosion, and no additional benefit could be obtained by the use of anti-erosive or repairing toothpastes.

As the composition of human saliva might show a high intra- and inter-sample variability and might be rapidly degraded or altered under in vitro conditions, artificial saliva was used in the present study which provides the advantage that it can be prepared in sufficient amounts and with a consistent composition, which leads to a high degree of standardisation.

Generally, all toothpastes were of lower efficacy under simulated intrinsic compared to extrinsic conditions. On the one hand, this observation might be explained by the very aggressive erosion regime, which might lead to a faster dissolution of possible surface precipitates. On the other hand, it is known that the efficacy of fluorides and tin chloride is affected by the presence of the organic matrix. In case that the erosively demineralised collagen matrix is degraded by pepsin or collagenase, the erosion-inhibiting effect of fluoride and tin compounds is reduced. The protective potential is then mainly related to the formation surface precipitates rather than to the incorporation of fluoride or tin in the organic matrix. [20,22,23]

Although several studies demonstrated that abrasion of eroded dentin depends strongly on the abrasivity of the toothpaste, [24,25,26,27,28] no clear impact of the RDA-value on dentin wear could be shown in the present study. It is for instance noteworthy that the fluoride-free toothpaste containing chitosan reduced dentin erosion by 40 to 60 % despite the very high RDA-value. Chitosan-containing toothpastes were already shown to be quite effective in reducing enamel erosion.[6,11]

Chitosan is a cationic polysaccharide, which is assumed to form acid-resistant multilayers. Moreover, due to its lubricating effects it might reduce the abrasivity of toothpastes by binding to the silica particles or the tooth surface.[11] Therefore, chitosan might also enhance the effect of stannous-fluoride containing toothpastes, which were shown to have a distinct anti-erosive effect on enamel.[7,11]

In case of eroded dentin, and especially when the organic matrix is not fully removed, tin is bound in the collagen layer and thus, led to an increased acid resistance compared to sodium fluoride.[22,3] This was also partly confirmed in the present study, as the toothpaste containing stannous-fluoride and chitosan showed a slightly better protective potential than the amine fluoride containing toothpaste under simulated extrinsic erosive conditions.

The fluoride-free product containing zinc-carbonate-hydroxyapatite nanoparticles (Biorepair) was least effective in the present experiment and also in an in vitro study analyzing the effect of toothpastes with special formulations on enamel erosion.[9] The significantly lower effect compared to the fluoride-containing toothpaste with nano-hydroxyapatite (Apacare) might be associated to the different abrasivity and the absence of fluoride in case of Biorepair. There is some evidence that hydroxyapatite nanoparticles might infiltrate demineralized dentin to provide a scaffold for remineralisation,[29,30] but only limited information is currently available about the interaction of nano-hydroxyapatite containing toothpastes with demineralized dentin. Apacare and Biorepair toothpastes were shown to be superior to an AmF toothpaste in terms of remineralization of artificial dentin caries lesions, but this effect was at least partly related to a more favorable pH value of the toothpastes with hydroxyapatite nanoparticles.[31] However, a positive effect of these toothpastes on dentin demineralization in pH-cycling experiments and/or under severe acidic conditions was not shown so far.

In conclusion, brushing with anti-erosive toothpastes significantly reduced dentin loss under simulated extrinsic or intrinsic erosive conditions, but was not superior to brushing with a conventional AmF toothpaste.

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Table 1. Manufacturer, composition and pH-values of the toothpastes

Toothpastes	Ingredients	LOT	pH
ApaCare **Cumdente GmbH**	Aqua, Hydrated Silica, Sorbitol, Propylene Glycol, Glycerin, Sodium C14-16 Olefin Sulfonate, Hydroxyapatite, Aroma, Cellulose Gum, CI 77891, Sodium Fluoride, Allantoin, Sodium Saccharin, Tetrapotassium Pyrophosphate, Limonene	AV1504	7.5
BioRepair **Dr.Kurt Wolff Forschung**	Aqua, Zinc Carbonate Hydroxylapatite, Hydrated Silica, Glycerin, Sorbitol, Silica, Aroma, Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Tetrapotassium Pyrophosphate, Zinc PCA, Cetraria Islandica Extract, Sodium Saccharin, Citric Acid, Phenoxyethanol, Benzyl Alcohol, Methylparaben, Propylparaben	228951114	7.9
Chitodent **B&F Elektro GmbH**	Aqua, Sorbitol, Hydrated Silica, Glycerin, Chitin/Chitosan, Acetic acid, Betaine, Paraffinum Liquidum, Titanium dioxide, Sodium saccharin	51922	6.0
Elmex **GABA**	Aqua, Hydrated Silica, Sorbitol, Hydroxyethylcellulose, (Olaflur) Amine fluoride, Aroma, Limonene, Titanium Dioxide, Saccharin	22051E	5.9
ELMEX Erosionsschutz **GABA**	Aqua, Glycerin, Sorbitol, Hydrated silica, Hydroxyethylcellulose, Aroma, Cocamidopropyl Betaine, Titanium dioxide, Olaflur, Sodium Gluconate, Stannous Chloride, Alumina, Chitosan, Sodium Saccharin, Sodium Fluoride, Potassium Hydroxide, Hydrochloric Acid	21311C	5.6
Mirasensitive **Hager Werken**	Aqua, Hydroxyapatite, Xylitol, Sorbitol, Propylene Glycol, Potassium Citrate, Tetrapotassium Pyrophosphate, Sodium C 14-16 Olefin Sulfonate, Disodium Pyrophosphate, Cellulose Gum, Aroma, Sodium Fluoride, Cocamidopropyl Betaine, Sodium Saccharin, Limonene, CI 77891,	080814	9.2
Sensodyne Proschmelz **GlaxoSmithKline**	Aqua, Sorbitol, Hydrated Silica, Glycerin, Potassium Nitrate, PEG-6, Cocamidopropyl Betaine, Aroma, Xanthan Gum, Sodium Saccharin, Sodium Fluoride, Titanium Dioxide, Sodium Hydroxide, Limonene, Anise Alcohol.	381C	7.4

Table 2. RDA-values (mean ± standard deviation) of the toothpastes and dentin loss (µm, mean ± stand deviation) at the end of the experiment. Within simulated extrinsic or intrinsic conditions, respectively, signific differences between groups were marked by different small letters.

Toothpaste	RDA	Erosion with citric acid (extrinsic condition)		Erosion with hydrochloric acid/pepsin solution (intrinsic erosion)	
		Dentin loss (µm)	Significant reduction compared to control (%)	Dentin loss (µm)	Significant reduction compared to control (%)
None (control)	-	5.1 ± 1.0^{a}	-	12.9 ± 1.4^{a}	-
ApaCare	30.9 ± 4.0	1.9 ± 0.4^{c}	61.8	8.2 ± 1.8^{c}	36.5
BioRepair	183.6 ± 19.6	3.9 ± 0.7^{b}	23.1	$12.8 \pm 1.2^{a,b}$	n.s.
Chitodent	203.9 ± 19.4	2.0 ± 0.8^{c}	60.4	$10.2 \pm 2.6^{b,c}$	20.7
elmex	57.4 ± 5.3	$2.8 \pm 0.5^{b,c}$	44.8	8.1 ± 1.3^{c}	37.0
elmex Erosionsschutz	18.8 ± 3.0	1.7 ± 0.5^{c}	66.8	7.8 ± 1.6^{c}	39.9
Mirasensitive	24.2 ± 4.4	2.8 ± 1.0^{c}	45.8	10.0 ± 1.3^{c}	22.2
Sensodyne Proschmelz	19.3 ± 2.2	2.0 ± 0.8^{c}	60.9	8.3 ± 1.3^{c}	35.7